American Journal of Medical Genetics 63:438–446 (1996)

Juvenile Myoclonic Epilepsy in Chromosome 6p12-p11: Locus Heterogeneity and Recombinations

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We recently analyzed under homogeneity a large pedigree from Belize with classic juvenile myoclonic epilepsy (JME). After a genome wide search with 146 microsatellites, we obtained significant linkage between chromosome 6p markers, D6S257 and D6S272, and both convulsive and EEG traits of JME. Recombinations in two affected members defined a 40 cM JME region flanked by D6S313 and D6S258. In the present communication, we explored if the same chromosome 6p11 microsatellites also have a role in JME mixed with pyknoleptic absences. We allowed for heterogeneity during linkage analyses. We tested for heterogeneity by the admixture test and looked for more recombinations. D6S272, D6S466, D6S294, and D6S257 were significantly linked ($Z_{max} > 3.5$) to the clinical and EEG traits of 22 families, assuming autosomal dominant inheritance with 70% penetrance. Pairwise Z_{max} were 4.230 for D6S294 ($\theta_{m=f}$ at 0.133) and 4.442 for D6S466 ($\theta_{m=f}$ at 0.111). Admixture test (H_2 vs. H_1) was significant (P = 0.0234 for D6S294 and 0.0128 for D6S272) supporting the hypotheses of linkage with heterogeneity. Estimated proportion of linked families, α, was 0.50 (95% confidence interval 0.05–0.99) for D6S294 and D6S272. Multipoint analyses and recombinations in three new families narrowed the JME locus to a 7 cM interval flanked by D6S272 and D6S257. © 1996 Wiley-Liss, Inc.

Received for publication June 16, 1995; revision received December 15, 1995.

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KEY WORDS: juvenile myoclonic epilepsy, heterogeneity, chromosome 6p11, recombinations

INTRODUCTION

The epilepsies are clinically variable and genetically heterogeneous. Juvenile myoclonic epilepsy (JME) [Delgado-Escueta and Bacsal, 1984] or "impulsiv petit mal" [Janz and Christian, 1957] is one of the common forms of idiopathic generalized epilepsies. It is characterized by adolescent myoclonic, grand mal tonic-clonic, or clonic tonic clonic seizures with or without absences. Electroencephalographs (EEGs) show high amplitude diffuse 8–20 Hz multispikes during myoclonic seizures while EEGs between seizures manifest the fast variety (3.5–6 Hz) of diffuse multispike wave complexes [Delgado-Escueta and Bacsal, 1984]. JME is an underdiagnosed disorder and is conservatively reported to involve at least 7% to 9% and perhaps as much as 20% of all epilepsies [Gooses, 1984; Janz 1985]. We think JME may be more common than reported. Grand mal epilepsy on awakening is reported to account for another 22% to 37% of all epilepsies [Tsuboi and Christian, 1976; Janz, 1969] but grand mal is rare as the sole manifestation of convulsive attacks. Grand mal seizures are preceded in 27% to 29% by myoclonic seizures [Janz, 1969; Tsuboi and Christian, 1976; Loiseau, 1964]. Thus, we infer that JME may account for as much as 20% to 30% of all epilepsies. JME is probably the most common form of idiopathic generalized epilepsy and the most common cause of primary grand mal seizures.

Because conflicting reports on the presence [Greenberg et al., 1988; Durner et al., 1991; Weissbecker et al., 1991] or absence [Whitehouse et al, 1993] of genetic linkage with chr.6p markers can be partially explained by the presence of interfamilial genetic heterogeneity, we recently studied a single large pedigree (38 members of four generations) with classic JME from a genetic isolate (Belize). In this family, five living and two

deceased affected members had JME but no pyknoleptic childhood absences. Four clinically asymptomatic females had EEG multispike wave complexes. We analyzed under homogeneity the results of a genome-wide search with 146 short tandem repeat polymorphic (STRPs) markers and found significant linkage ($Z_{max} > 3.3$, $\theta_{m=f}$ at 0.00) between D6S257 and D6S272 and both convulsive and EEG traits of JME [Liu et al., 1995]. In addition, we identified two informative recombinations in this large Belize pedigree and identified a 40 cM JME region flanked by D6S313 centromeric and D6S258 telomeric [Serratosa et al., 1995].

In this communication, we explore if the same chr. 6p11 STRPs also have a role in families whose JME may or may not be combined with pyknoleptic absences. We report the results of pairwise linkage analysis under assumption of heterogeneity. We also report results of the admixture test [Risch, 1988; Faraway, 1993] and multipoint linkage analyses. We describe phenotypic dissimilarities between linked and unlinked families, and identify more recombinations in three new families which narrow the JME region to 7 cM flanked by D6S272 telomeric and D6S257 centromeric.

MATERIALS AND METHODS

Twenty-two multiplex families (Fig. 1) were ascertained through patients being treated for JME with or without pyknoleptic absences. The affected relatives either had JME or grand mal tonic clonic or absence epilepsies. We examined 297 relatives and performed EEGs in 261 members of these 22 families who live in Los Angeles (United States of America), Mexico City (Mexico), Belize City, and Corozal (Belize). Seventeen of the twenty-two pedigrees were previously reported by Liu et al. [1995].

Criteria for inclusion of probands were expanded to include childhood pyknoleptic absences which evolved to JME (nine patients) and JME with sudden falls (one patient). Inclusion criteria consist of the following: 1) Myoclonic seizures must be present. They must start between 8 to 20 years of age, usually on awakening involving shoulders, arms and other parts of the limbs. Myoclonic seizures must not be associated with loss of consciousness. 2) Tonic clonic or clonic tonic clonic convulsions may be present at onset or appear 1 to 10 years after the start of myoclonic seizures. 3) Childhood pyknoleptic absences starting as early as 3 years of age or juvenile spanioleptic absences starting at 8 to 16 years of age may be the presenting symptom/sign. However, they must evolve into JME during adolescence. 4) Neurological examination, including mental status and intelligence must be normal, and 5) diffuse synchronous and symmetrical 3.5–6 Hz polyspike-wave complexes must be present in the interictal EEG. Some patients also have EEG 2.5 to 3.5 Hz diffuse spike and wave complexes, characteristic of childhood absence.

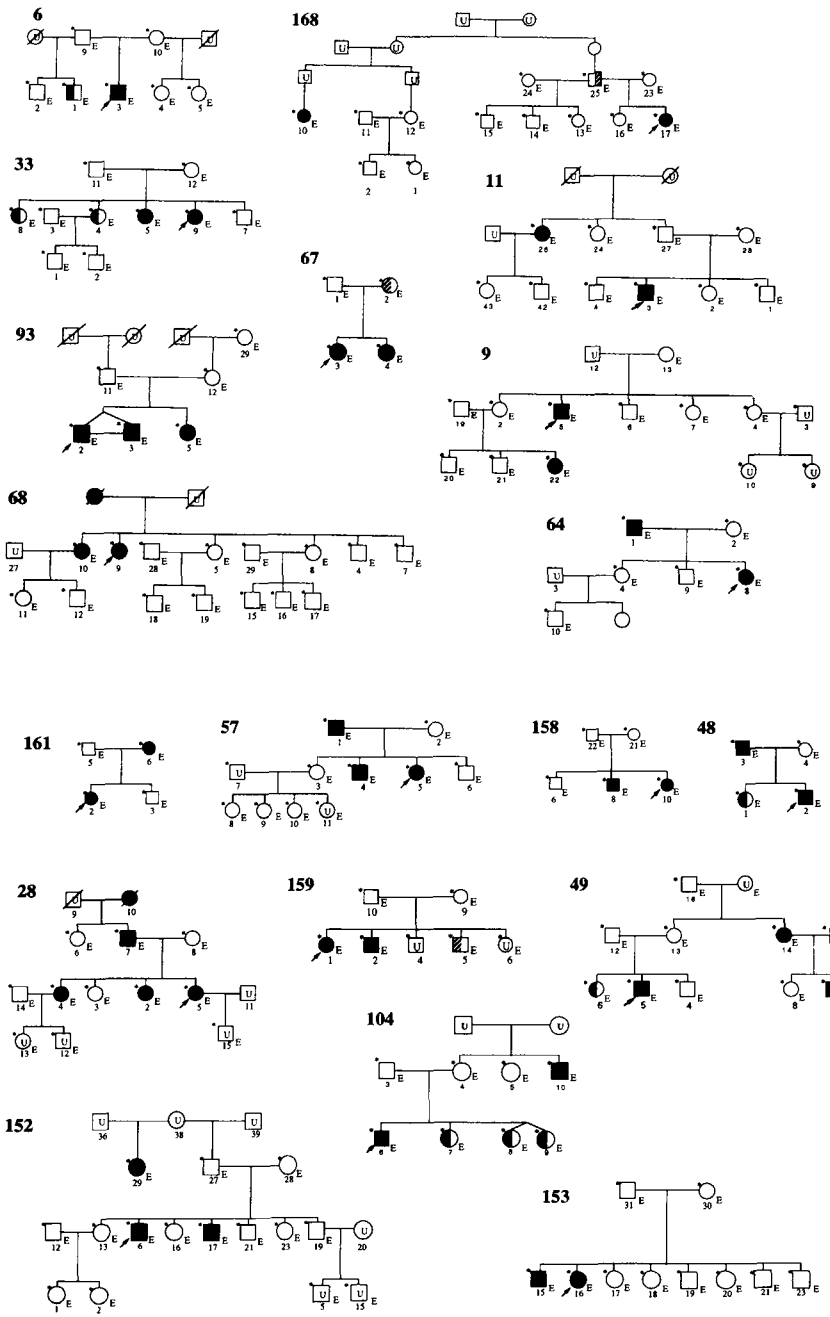
Exclusionary criteria are 1) structural lesions in the CNS and metabolic or degenerative diseases which manifest as myoclonic seizures, 2) myoclonic seizures which are only stimulus sensitive as in progressive myoclonus epilepsies, 3) myoclonic absences and myoclonus absences, 4) partial seizures, 5) tonic seizures, and 6) childhood pyknoleptic absences with 3 Hz spike wave complexes as the only seizure type in the proband.

We used only one diagnostic model during linkage analyses. In this diagnostic model, clinically asymptomatic relatives with epileptogenic EEG paroxysms (diffuse 3.5–6 Hz multispike wave complexes or 2.5–3.5 Hz spike-wave complexes) were classified as affected, in addition to relatives clinically affected with epilepsies. Individuals with febrile convulsions during infancy or early childhood were considered unaffected. Four relatives under 8 years of age (9-10, 57-11, J1-19, and J1-127) and relatives who did not have EEGs were classified as "unknown" during linkage analysis. Fifty-six relatives were clinically affected with epilepsy. Fifteen relatives (3 males and 12 females) were clinically asymptomatic but had epileptogenic 2.5–3.5 Hz spike wave complexes (4 relatives) or 3.5–6 Hz multispike wave complexes (11 relatives) in their EEGs. Five individuals (two males and three females) had nonspecific but epileptiform bursts of diffuse spike and sharp wave formation in their EEGs. Forty-one individuals in 13 pedigrees were under 19 years of age and were taken into consideration during correction for age-dependent penetrance in linkage analysis.

We (M.T.M., A.V.D.-E., and S.C.) independently assessed each member of the families in Mexico City, collected the clinical and family information and interpreted EEGs. We (M.T.M. and A.V.D.-E.) did the same for relatives of patients in Los Angeles (CA) and Corozal (Belize). These factors were used to determine the syndromic classification of each relative while MNG and JMS. collated these results to see if phenotypic differences existed between linked and unlinked families. Criteria for syndromic classification were described by Delgado-Escueta et al. [1983] and the International League Against Epilepsy [1985].

Linkage analyses were carried out using the LINKAGE program package, version 5.10 for PC [Ott, 1974; Lathrop et al., 1984]. Pairwise lod scores were estimated by MLINK assuming equal rates of male and female recombination frequencies. The pattern of inheritance of JME was assumed to be dominant with 70% penetrance. The gene frequency for the JME allele was assumed as 0.001. Analyses were undertaken with and without corrections for age-dependent penetrance. Based on our age-onset study in 100 JME probands, six liability classes for age-dependent penetrance were incorporated into the linkage analysis. The corresponding penetrances for each liability class are listed in the following: 1) under 10 years old, 4%; 2) ages 11 to 12, 10%; 3) ages 13 to 14, 25%; 4) ages 15 to 16, 50%; 5) ages 17 to 18, 60%; and 6) ages 19 and older, 70%.

Evidence of heterogeneity was evaluated using HOMOG program version 3.33. HOMOG program [Ott, 1991] was used to test the significance among three hypotheses: H_0, the hypothesis of no linkage in any family; H_1, the hypothesis of linkage in all families; and H_2, the hypothesis of linkage in only a subset of families. HOMOG generates the likelihood of the data under each hypothesis, allowing the use of the likelihood-ratio test. The resulting -2ln (likelihood) is approximately distributed as a χ^2 with df equal to the difference in the number of



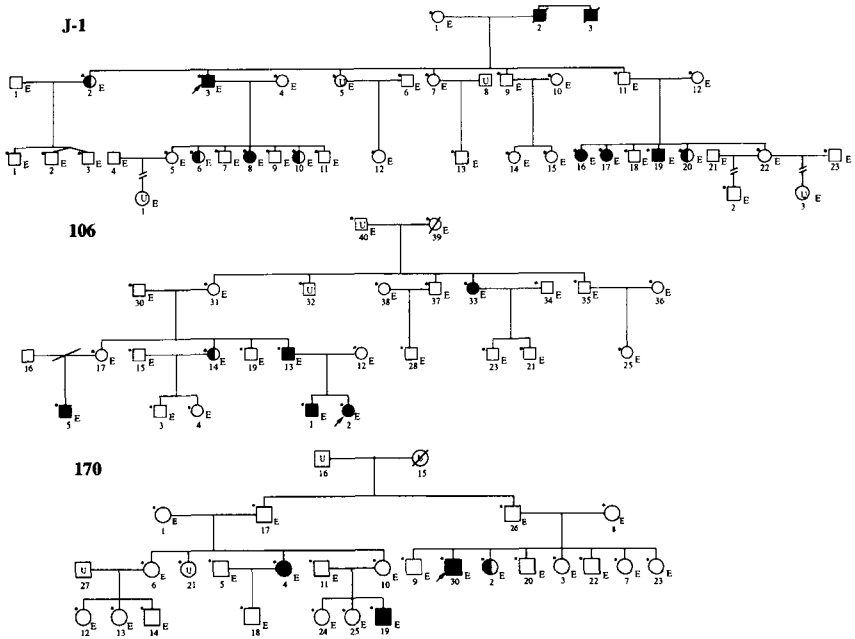


Fig. 1. 22 JME pedigrees. The symbols ● ■ represent clinically affected persons. The symbols ◐ ◧ refer to clinically asymptomatic family members whose EEGs show 3.5–6 Hz multispike wave complexes and/or 3 Hz spike wave complexes. The symbols ◴ ◨ refer to clinically asymptomatic persons whose EEGs show non-specific but epileptiform bilateral and diffuse 4–7 Hz spike and sharp wave formations. The symbols '*' means DNA is available. The symbol 'E' means EEG was performed. The symbol 'U' means the person was considered unknown with regards to their state of affectedness during linkage analyses.

parameters estimated. Heterogeneity is suspected if the H_1 vs. H_2 test is significant.

DNA genotyping was done in the following manner. Blood samples were obtained by venipuncture from consenting relatives. High-molecular-weight genomic DNA was isolated from 10 ml of peripheral blood [Sambrook et al., 1989] which we collected from 254 relatives. Using the method of Weber et al.[1989], nucleotide repeat polymorphisms were typed by PCR amplification using flanking oligonucleotide primers. STRP DNA markers D6S89 [Litt et al., 1990], D6S105 [Weber and May, 1991], D6S89, TNF (tumor necrosis factor) [Nedospasov et al., 1991], D6S282, TCTE1 [Kwiatkowski et al., 1991], D6S426, D6S271, D6S272, D6S295, D6S294, D6S257, D6S452, D6S466, D6S402, and D6S430 [Weissenbach et al., 1992; Gyapay et al., 1994] were chosen for genotyping. All the sequences and allele frequencies of STRP DNA markers are available from the Genome Data Base (GDB). A diagram of chr. 6p with STRP markers is shown in Figure 2.

RESULTS

Pairwise Linkage Analyses in 22 JME Pedigrees Support Linkage to chr. 6p11 STRPs

Results of pairwise linkage analysis between JME in 22 families and 12 markers in chr. 6p are presented in Table I, under the assumptions of heterogeneity and autosomal dominant inheritance with 70% penetrance. In contrast to the earlier reports by Durner et al. [1991] and Greenberg and Delgado-Escueta [1993], which suggested linkage to serologic HLA-BF and DNA markers in the HLA region, summed lod scores were negative (−25.472 to −2.819 at $\theta_{m=f}$, 0.00–0.100) for TNF. The TNF locus resides within the HLA region and is approximately 500 Kb telomeric from BF [Udalova, et al., 1993]. Summed Z_{max} values in 22 families exceeded the threshold (>3.8) for significance [Risch, 1988; Faraway, 1993] for D6S272, D6S466, D6S294, and D6S257 covering 7 cM. The highest summed lod scores, namely 4.442 ($\theta_{m=f}$ at 0.111) and 4.230 ($\theta_{m=f}$ at 0.133), were obtained for D6S466 and D6S294, respectively. We did not observe any major differences in the lod score values when we corrected for age-dependent penetrance. When age-penetrance was taken into consideration, summed lod score for D6S466 and D6S294 were 4.432 ($\theta_{m=f}$ at 0.1) and 4.371 ($\theta_{m=f}$ at 0.1), respectively.

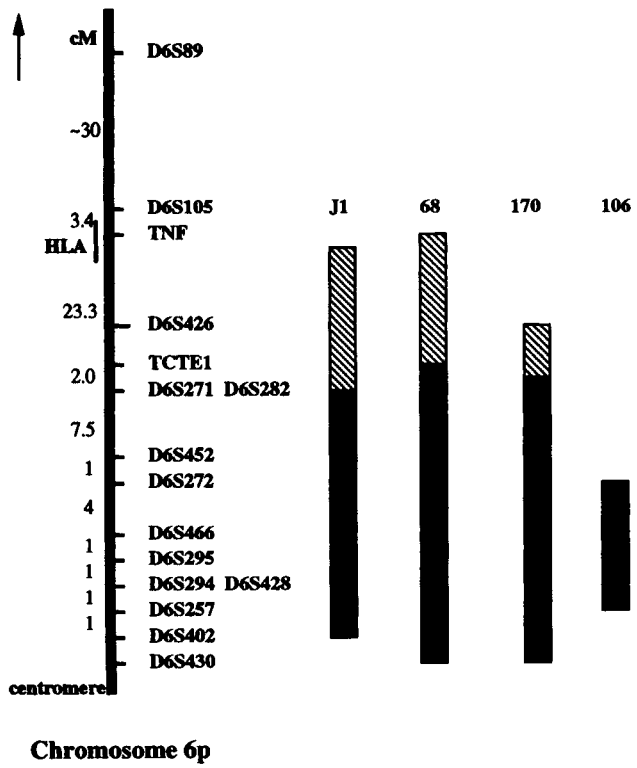


Fig. 2. A chromosome 6p map and recombination events in pedigrees J1, 68, 170, and 106. Shaded areas represent the regions where recombination occurred. Dark bars indicate the candidate JME region in each pedigree.

HOMOG Indicates Heterogeneity

Assuming autosomal dominant inheritance with 70% penetrance (no age correction), we tested for homogeneity using pairwise lod scores for D6S257, D6S294, and D6S272. H_2 vs. H_1 showed significance ($P = 0.0234$ for D6S294 and $P = 0.0128$ for D6S272) supporting the hypothesis of linkage with heterogeneity and providing an estimated (α) 0.5 (95% confidence interval 0.05–0.99) for the proportion of chr.6p linked families in our pedigrees (Tables II and III).

Exclusion of Linkage to chr. 6p11 STRPs by Pairwise lod Scores and Haplotypes in Four Families Also Support Hypothesis of Heterogeneity

The epilepsy and EEG traits of family 9 and family 104 are unlikely to be linked to D6S257, D6S272, and D6S294 (Table III) because the posterior probability of linkage, W*i* was less than 0.25 for family 9 and less than 0.09 for family 104. Pedigree 11 and pedigree 49 were uninformative for D6S272, but these two pedigrees showed low probabilities of linkage (<0.3) for D6S294 and D6S257. In addition, lod scores (<−2) were exclusionary for chr.6p11 markers D6S257 and D6S294 in pedigrees 9, 11, 49, and 104. Examination of haplotypes in these 4 pedigrees also suggested exclusion of linkage for the JME candidate region in chr. 6p11. In pedigree 9, individual 9-22 was affected with early childhood absences and grand mal. Her mother 9-2 is presumed to be an asymptomatic carrier of the epilepsy trait. Individuals 9-2

and 9-22 had entirely different haplotypes when compared to the proband 9-5. In pedigree 11, the proband (11-3) did not share any common alleles with the other clinically affected member (11-26). In family 104, two clinically affected members (104-10 and 104-6) as well as three asymptomatic individuals who had epileptogenic EEG trait did not share a common haplotype. Likewise, we could not detect any common alleles which segregated with epilepsy among the affected individuals in pedigree 49. These observations agree with the significantly low posterior probability of linkage or W*i* values for D6S257 and D6S294 in these four pedigrees. Thus, exclusion of linkage to chr. 6p11 STRPs by

TABLE I. Summed Pairwise lod Scores for 12 Microsatellites in Chromosome 6p for JME Families Ascertained Through JME With or Without Absences

Markers	θ_{m-f}								$Z_{max}(\theta)$
Locus symbol	0.000	0.001	0.010	0.050	0.100	0.200	0.300	0.400	
D6S89	−22.986	−20.386	−15.380	−8.196	−4.618	−1.474	−0.266	0.080	0.088 (0.421)
D6S105	−25.075	−18.786	−11.356	−4.287	−1.176	1.093	1.399	0.821	1.432 (0.274)
TNF	−25.472	−20.042	−12.753	−5.986	−2.819	−0.121	0.581	0.431	0.597 (0.321)
TCTE1	−17.094	−12.634	−6.859	−1.804	0.294	1.729	1.695	0.981	1.832 (0.244)
D6S271	−13.944	−9.699	−4.380	0.312	2.152	2.991	2.351	1.141	2.996 (0.192)
D6S282	−13.161	−9.007	−4.366	−0.488	1.192	2.316	1.995	1.057	2.334 (0.216)
D6S272	−5.604	−4.061	−1.290	2.106	3.327	3.510	2.580	1.243	3.638 (0.157)
D6S466	−1.222	−0.225	2.063	3.962	4.430	3.935	2.680	1.203	4.442 (0.111)
D6S294	−4.623	−2.992	0.100	3.135	4.104	3.890	2.614	1.015	4.230 (0.133)
D6S257	−7.778	−5.067	−1.287	2.263	3.537	3.708	2.704	1.297	3.853 (0.155)
D6S402	−11.063	−8.298	−4.052	0.336	2.186	3.058	2.459	1.243	3.059 (0.195)
D6S430	−16.577	−12.778	−7.691	−2.605	−0.306	1.276	1.313	0.703	1.414 (0.251)

pairwise lod score and haplotype analyses in these four families provides further evidence in support of the hypothesis of heterogeneity.

Multipoint Linkage Analyses Suggest a 7 cM Area Spanned by D6S272-(D6S466-D6S294)-D6S257

To minimize systematic bias in estimates of the recombination fraction during linkage analyses under locus heterogeneity, the best estimates for θ are obtained in multipoint analysis, especially if misclassifications or family size differences are present [Ott, 1991, Janssen et al., 1995].

We used 11 markers (D6S89, D6S105, TNF, TCTE1, D6S271, D6S272, D6S466, D6S294, D6S257, D6S402, and D6S430) for multipoint linkage analysis in the 11 linked families. The genetic distance between STRPs used were based on the genetic linkage map published by Genethon [Gyapay et al., 1994]. The lod score curve led to the highest peak value of 14.576 between D6S466 and D6S294 (Fig. 3). According to the ($Z_{max} - 1$) method [Conneally et al., 1985], the JME locus is probably in the region spanned by D6S272-(D6S466-D6S294)-D6S257, a 7 cM space.

Recombinations in Three New Families Narrow the JME Region Further to the Same 7 cM Area Suggested by Multipoint Analyses

We had previously reported a genetic recombination between D6S276 and D6S282 in one clinically affected member (III-8) of a large LA-Belize family afflicted with classic JME [Liu et al., 1995]. We also observed one recombination between D6S313 and D6S467 in one other relative (III-19) who is EEG affected. These two recombinations had positioned the JME locus to a 40 cM interval with flanking markers D6S313 centromeric and D6S258 telomeric [Serratosa et al., 1995]. We also observed in this large family a cross-over between D6S271 and D6S269 in an unaffected adult which further suggested that the JME locus may be centromeric to D6S271 [Liu et al., 1995].

In our present report, recombinations in three new families narrowed the JME region. A recombination between TNF and TCTE1 in one affected member (68-9) of family 68 agrees with previous observations on recombinations in the LA-Belize family, suggesting a 40 cM JME region. A recombination between D6S426 and TCTE1 in two affected persons (170-30 and 170-2) of family 170 placed the JME region below D6S426 which is 11 cM below HLA. This reduced the size of the JME region to 28 cM. Four clinically affected members and one EEG affected member in pedigree 106 shared the same haplotype which included D6S466-D6S295-D6S294 (D6S428) (Fig. 1). Crossing-over between D6S428 (or D6S294) and D6S257 in three clinically affected individuals placed the JME region above D6S257, providing a centromeric flanking marker. Another recombinant event between D6S466 and D6S272 in one clinically affected member (106-5) suggested a JME region below D6S272, providing a telomeric flanking marker. Recombinant events in family 106, therefore, further narrowed the JME region to 7 cM flanked by D6S272 on the telomeric side and D6S257 on the centromeric side.

TABLE II. Tests of Linkage Homogeneity*

Marker	D6S272		D6S294		D6S257	
Test (df)	χ^2	P	χ^2	P	χ^2	P
H_2 vs. H_1 (1)	6.197	0.0128	5.141	0.0234	2.864	0.090
H_1 vs. H_0 (1)	15.602	<0.0001	18.890	<0.0001	19.305	<0.0001
H_2 vs. H_0 (2)	21.799	<0.0001	24.032	<0.0001	22.169	<0.0001

*H_0, the hypothesis of no linkage in any families; H_1, the hypothesis of linkage in all families; and H_2, the hypothesis of linkage in only a subset of families.

TABLE III. Posterior Probability of Linkage (W*i*) to Markers D6S272, D6S294, and D6S257 for Each of the 22 Families Assuming Autosomal Dominant Transmission With 70% Penetrance

Alpha (Theta)	D6S272 0.50 (0.000)	D6S294 0.50 (0.00)	D6S257 0.60 (0.050)
		(W*i*) Probability	
6	0.717	0.754	0.801
9	0.176	0.001	0.221
11	0.512*	0.003	0.262
28	0.002	0.004	0.683
33	0.897	0.897	0.859
48	0.666	0.666	0.730
49	0.514*	0.002	0.169
57	0.490*	0.850	0.876
64	0.720	0.720	0.772
67	0.002	0.334	0.472
68	0.682	0.742	0.744
93	0.625	0.630	0.693
104	0.002	0.007	0.085
106	0.808	0.860	0.912
152	0.000	0.499	0.225
153	0.450	0.375	0.491
158	0.500	0.188	0.459
159	0.176	0.490	0.353
161	0.606	0.606	0.683
168	0.080	0.376	0.407
170	0.968	0.992	0.964
J1	1.000	1.000	1.000

*Families 11, 49, and 57 were not informative for marker D6S272 (lod score ~ 0). Age corrections did not make any notable difference in Wi.

Dissimilarities in Phenotype May Exist Between Linked and Unlinked Families

We also analyzed the clinical manifestations and EEG traits of each clinically affected and EEG affected member of chr. 6p linked and unlinked families. We performed the Fisher exact test of independence between *Wi*, the posterior probability of linkage, and 1) the presence of 3 Hz spike and wave complexes as the EEG signature of childhood pyknoleptic aabsences and 2) the presence of pyknoleptic absences, which may appear in early childhood, childhood, adolescence, or adulthood. Pyknoleptic absences, although more commonly associated with 3 Hz spike-wave complexes, can also be observed with rapid 4–6 Hz polyspike wave complexes or 12 Hz diffuse rhythms [Delgado-Escueta et al., 1983]. The results are shown in Table IV(A) and IV(B). Four of nine individuals in families with low posterior probability of linkage, or $Wi < 0.3$ show 3 Hz spike-wave complexes, whereas only 1 of 38 persons in families with high posterior probability of linkage, or $Wi > 0.6$ does. When *Wi* falls below 0.3, 3 Hz spike-wave complexes were more likely to be present, ($P = 0.011$, two tail Fisher exact test). Similarly, though not as significant, we observed that five of eight individuals in families with low posterior probability of linkage, or $Wi < 0.3$ have clinical absences; whereas only five of 34 individuals in families with high posterior probability of linkage, or $Wi > 0.6$ have clinical absences, ($P = 0.097$, two tail Fisher exact test). In seven families, values of *Wi*, the posterior probability of linkage, did not resolve the issue of whether linkage was present or not, because STRPs or family material were uninformative.

DISCUSSION

Absences are commonly observed in JME and occur in 10% to 38% of JME patients [Janz, 1985; Panayiotopoulos et al., 1989; Mai et al., 1990]. Since pyknoleptic absences, which characteristically occur 1 to 200 times per day are observed in only 5% of JME, most absences in JME patients are presumed to be rare and random or spanioleptic in type. Interestingly, 4% to 8% of patients with JME evolve from pyknoleptic childhood absences [Janz, 1985]. Nine of our twenty-two families reported childhood pyknoleptic absences. We verified such seizures with their characteristic EEGs, namely the regular and well formed 3 Hz single spike and slow wave complex in seven of these nine families. Seven other families reported rare, random, spanioleptic juvenile absences. We documented that they did not have the characteristic EEG of childhood absence epilepsy. All in all, 16 of 22 families reported some form of absences.

Only a few patients with JME fall from a massive jerk. Obeid and Panayiotopoulos [1989] observed sudden falls in 2 of 50 patients while Salas-Puig [1988] noticed sudden falls and drops in seven of 20 patients. We observed falls in only one of our 22 patients.

Most reports on JME do not show any sex preference but Tsuboi and Christian [1976] and Tsuboi [1977] showed more female relatives were symptomatic with epilepsy. Canevini et al. [1992] also noted a high percentage of female patients, while a slightly increased prevalence among females was also noted by Asconape and Penry [1984] and Salas-Puig et al. [1988]. We did not find female preponderance among probands (11 females, 11 males) or among clinically affected relatives (30 females, 26 males). However, we did observe that clinically asymptomatic females were more frequently affected by EEG polyspike wave abnormalities than males (12 females, 3 males).

Using the above families of JME patients, we tested for linkage under the assumption of heterogeneity and obtained significant linkage with D6S466 and D6S294 ($Z_{max} > 4$, $\theta_{m=f}$ at 0.111 and $\theta_{m=f}$ at 0.133, respectively). Although lod scores derived under heterogenous models (Z, $\alpha\theta$) where $Z(\alpha,\theta) = \log \alpha \bullet 10^{Z(\theta)} + (1-\alpha)$ have an additional degree of freedom ($\alpha \neq 1.0$), a lod score of 3 is suggestive but not sufficient. Risch [1988] proposed threshold values of 3.7 or 3.8 assuming the admixture test has an asymptotic chi square distribution while Faraway [1993] concluded that a threshold of 3.3 would be appropriate. Our results provided sufficient and significant lod score, ($Z_{max} > 3.8$) for three STRPs in chr.6p11, namely, D6S466, D6S294, and D6S257.

To test for heterogeneity in our JME pedigrees, linkage results were further analyzed by the admixture test using the HOMOG computer program [Ott, 1991]. An α of 0.5 at $\theta_{m=f}$, at 0.00 indicated that 50% of our families were linked to D6S294 and D6S272. Our present families also identified four of 22 families who had a very low conditional probability of linkage ($Wi < 0.008$)

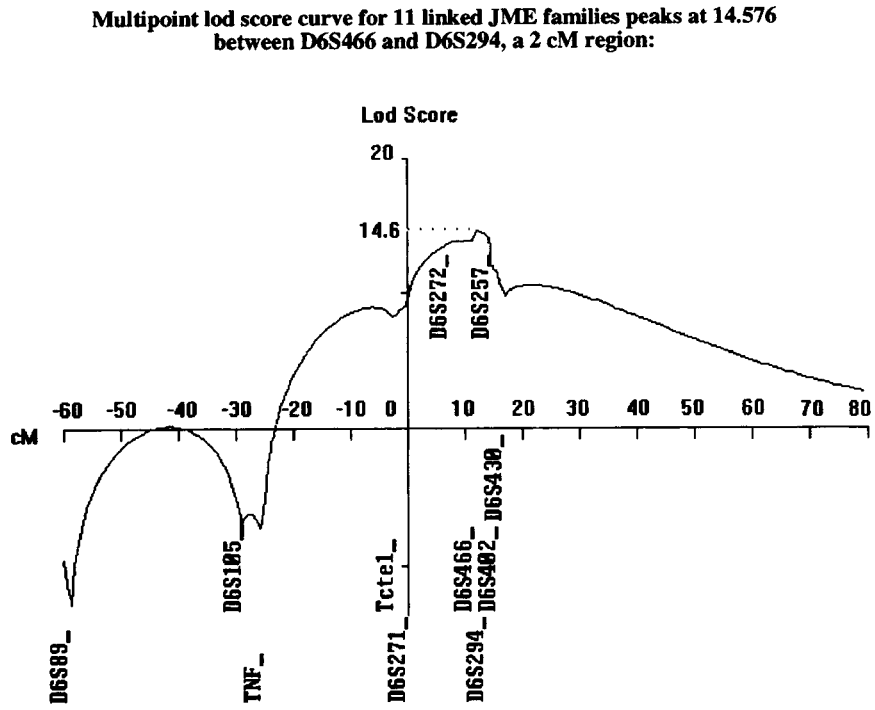


Fig. 3. Multipoint analyses: lod scores vs. map distance in cM.

for D6S294. When lod score for exclusion [<−2.0 for D6S294 at ($\theta_{m=f}$, at 0.0–0.001)] was applied to these same four families (families 9, 11, 49, and 104), linkage to the 90 cM region between D6S89 and D6S430 was excluded by multipoint linkage analyses.

To help determine the correct θ estimate, multipoint analyses was performed and yielded lod scores which peaked at 14.576 in a 2 cM area between D6S466 and D6S294. According to the (Z_{max} − 1) method of Conneally et al. [1985], the JME region is a 7 cM interval spanned by D6S272 and D6S257.

When using crossover information for physical mapping of a disease gene region, it is necessary to make sure that the families used are of the linked type. In our present study, HOMOG identified four families with high posterior probability of linkage ($Wi > 0.6$) who had informative recombinations. However, only one of these families had $Z_{max} > 3.3$, while three others had Z_{max} between 0.4 and 2.2. Ideally, informative recombinations would be most useful in families whose lod scores are over 3.3. With these limitations in mind, we did identify recombinations in four families whose Wi were greater than 0.6. Recombinations in affected members of these four families defined a 7cM JME region flanked by D6S272 on the telomeric side and D6S257 on the centromeric side. This is the same 7 cM region suggested by multipoint linkage analyses.

We compared the clinical manifestations of families who had high posterior probability of linkage ($Wi > 0.6$) against families with low posterior probability of linkage ($Wi < 0.3$) with reference to chr. 6p11 markers. The two tail Fisher exact test ($P = 0.011$) indicated that when Wi falls below 0.3, EEG 3 Hz spike wave complexes were more likely to be present. We also observed a similar though not as significant trend with regards to the presence of clinical absences. Nine probands of the 11 families linked to chr. 6p11 markers had the same electroclinical phenotype, namely classical JME without pyknoleptic absences. However, affected members of two other families (pedigrees 6 and 106) with high posterior probability of linkage started with childhood pyknoleptic absences and 3 Hz spike wave complexes that eventually developed into JME. These latter phenotypes were similar to the phenotypes of all four families which showed exclusionary lod scores in

TABLE IV. A) Fisher Exact Test for the Independence Between Wi, the Posterior Probabiligy of Linkage, and 3 Hz Spike-Wave Complexes EEG Trait

	Number of persons with 3 Hz S-W complexes	Number of persons without 3 Hz S-W complexes
$Wi < 0.3$	4	9
$Wi > 0.6$	1	38
Fisher exact test (2 tail): $P = 0.011$		

B) Fisher Exact Test for the Independence Between Wi, the Posterior Probability of Linkage, and Presence of Pyknoleptic Absences

	Number of persons with pyknoleptic absences	Number of persons without pyknoleptic absences
$Wi < 0.3$	5	8
$Wi > 0.6$	5	34
Fisher exact test (2 tail): $P = 0.097$		

that childhood pyknoleptic absences and 3 Hz spike wave complexes were present in probands and relatives.

Thus, our present data suggest that families with classical JME and the fast EEG 3.5–6 Hz polyspike wave complexes are significantly linked to chr. 6p11 STRPs and that an epilepsy locus exists between D6S272 and D6S257. Our present data also suggest that rare families with childhood absence which evolves to JME could be allelic variations [Romeo et al., 1994] of the chr. 6p11 JME locus. In addition and equally important, our present data do suggest that most families with JME combined with childhood pyknoleptic absence and EEG 3 Hz spike wave complexes do not map to the JME chr. 6p11 locus, supporting the notion of locus heterogeneity.

Aside from explaining phenotypic diversity, we also have to explain why female individuals are more at risk to develop the EEG polyspike wave trait (13 such females in seven of 22 families) and why low penetrance are common findings. A mutation in a second gene of the suppressor type in the X chromosome may make females at increased risk for the EEG polyspike trait. In ten of 22 JME families, several offspring were affected with JME, but not their parents. Germ-line mosaicism in parents is one possible explanation for the presence of disease in children but not in parents [Bernards et al., 1994] (see pedigrees 6, 93, and 33). The existence of a mutation in a fraction of the parents' germ-line may explain the affected status in their children. Germ-line mutations have been confirmed in several autosomal dominant neurological disorders such as neurofibromatosis-type I [Lazaro et al., 1994], Duchenne muscular dystrophy [Pegoraro et al., 1994; Bunyan et al., 1994] and molytic hyperkeratosis [Paller et al., 1994].

In conclusion, our present data on 22 JME families identified new recombinations in affected members of three new families narrowing the JME region to 7 cM flanked by D6S272 and D6S257. Our present results provide the first statistical evidence that genetic heterogeneity is present in the autosomal dominant form of JME. Because genetic heterogeneity can be a confounding factor in positional cloning, further refined linkage mapping in JME may be best carried out in larger and more densely affected families from genetic isolates. In doing so, it is necessary to be cautious that other epilepsy susceptibility genes are not introduced by marriage into these larger families.

ACKNOWLEDGMENTS

We thank our patients and their families for their cooperation and participation. This study was supported primarily by NIH Program Project 1P01 NS21908 (AVDE) and in part by a special medical program (AVDE) of the VA Southwest Regional Epilepsy Center at the West Los Angeles VA Medical Center.

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